

The logo for QIIME2, featuring a stylized grey tree structure with a red node, a green node, and a blue node, positioned above the text "qiime2".

qiime2 Platform

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An Introduction to QIIME2

Cancer and the microbiome

What is QIIME2?

QIIME2 Core Installation

Tools for reproducibility and visualization

Showcase of core methods and visualizations

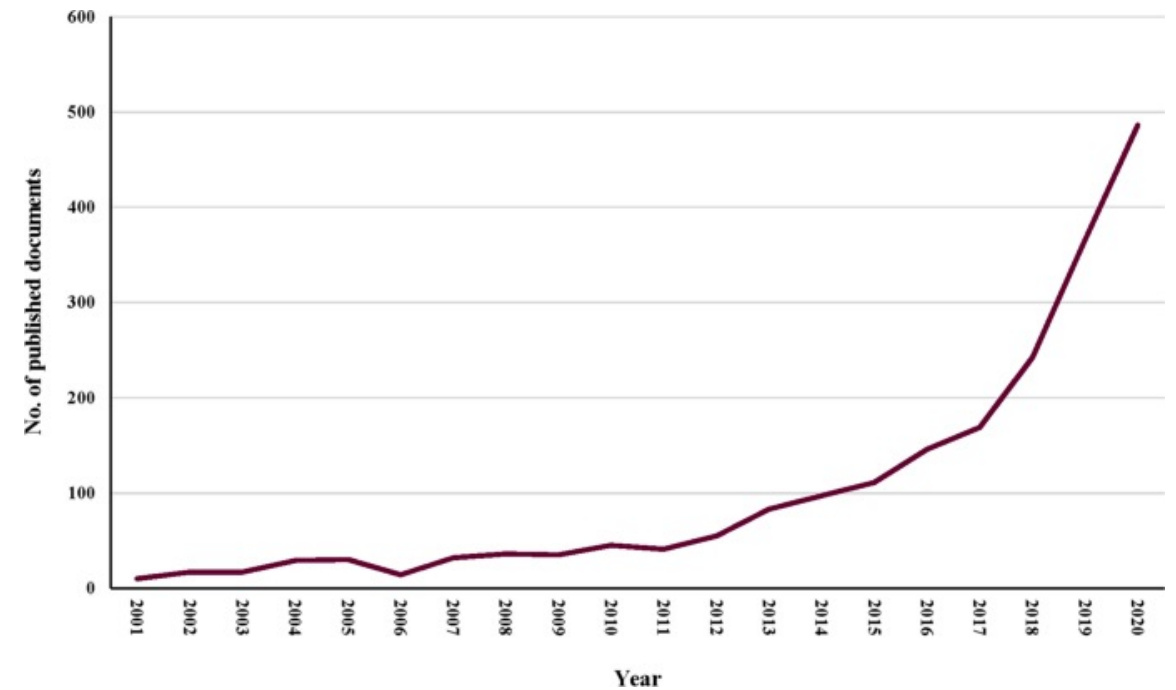
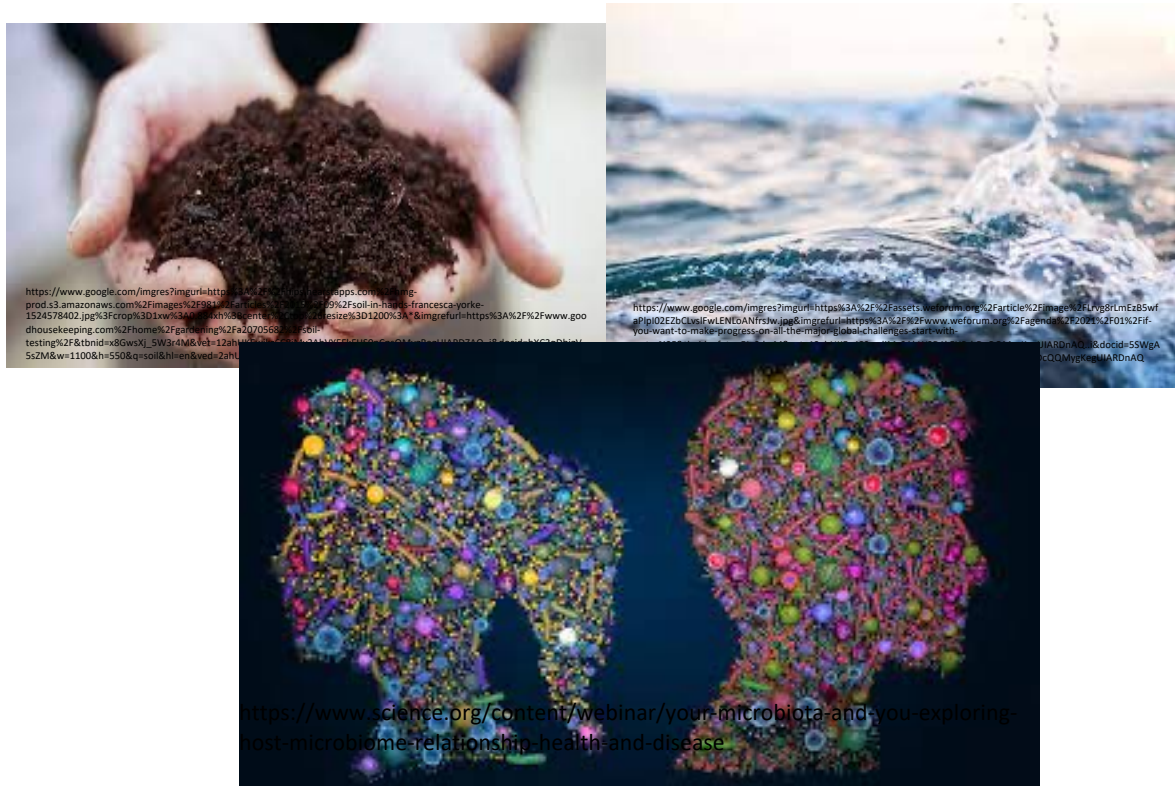
Community Support

Workshops and tutorials

The future of QIIME2

Trends in Microbiome Research

Microbiome – a collection or community of microorganisms in a defined environment



Growth trends of publications on the microbiota and cancer from 2001 to 2020, Zyoud et al. 2022, *Journal of Translational Medicine*

The role of the microbiome in cancer

The microbiome, cancer, and cancer therapy

[Beth A. Helmink](#), [M. A. Wadud Khan](#), [Amanda Hermann](#), [Vancheswaran Gopalakrishnan](#) & [Jennifer A. Wargo](#) 

[Nature Medicine](#) **25**, 377–388 (2019) | [Cite this article](#)

52k Accesses | **321** Citations | **240** Altmetric | [Metrics](#)

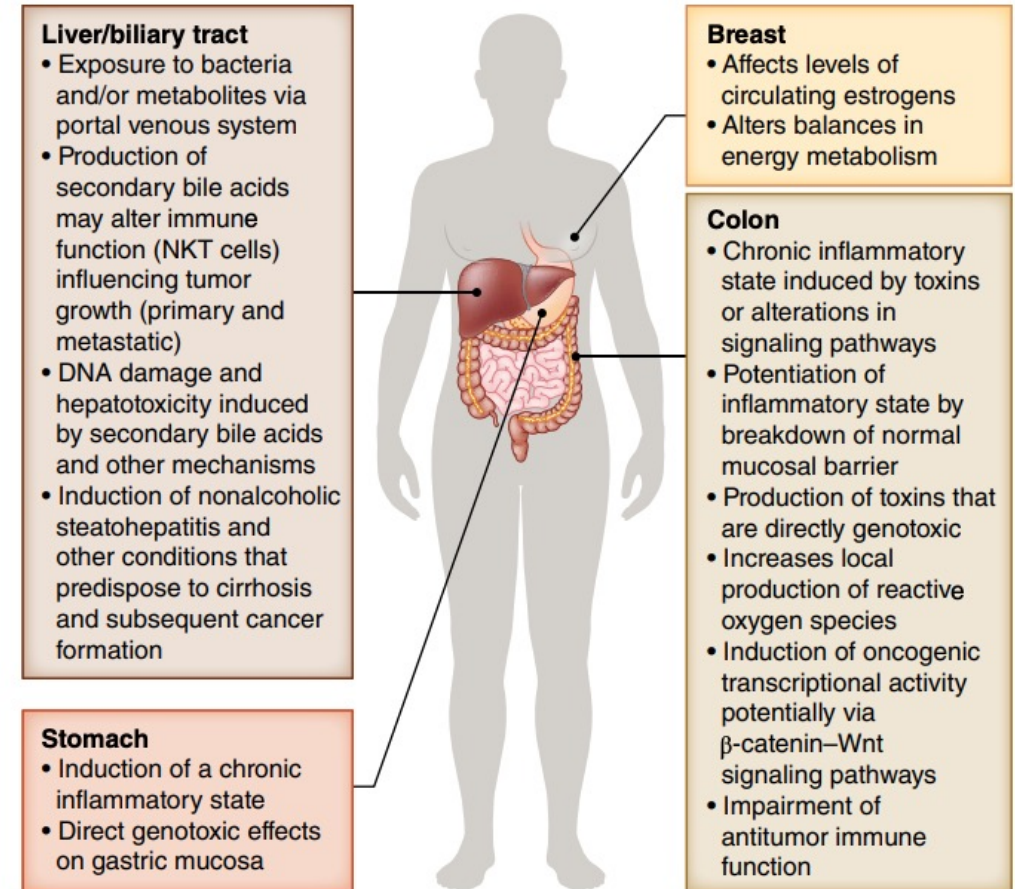


Fig. 1 | The influence of gut microbiota on cancer development. Shown are various mechanisms through which dysbiosis is proposed to affect tumorigenesis and/or tumor growth across cancer types, including colon, hepatobiliary, gastric and breast. Credit: Debbie Maizels/Springer Nature.

The role of the microbiome in cancer

The microbiome, cancer, and cancer therapy

[Beth A. Helmink](#), [M. A. Wadud Khan](#), [Amanda Hermann](#), [Vancheswaran Gopalakrishnan](#) & [Jennifer A.](#)

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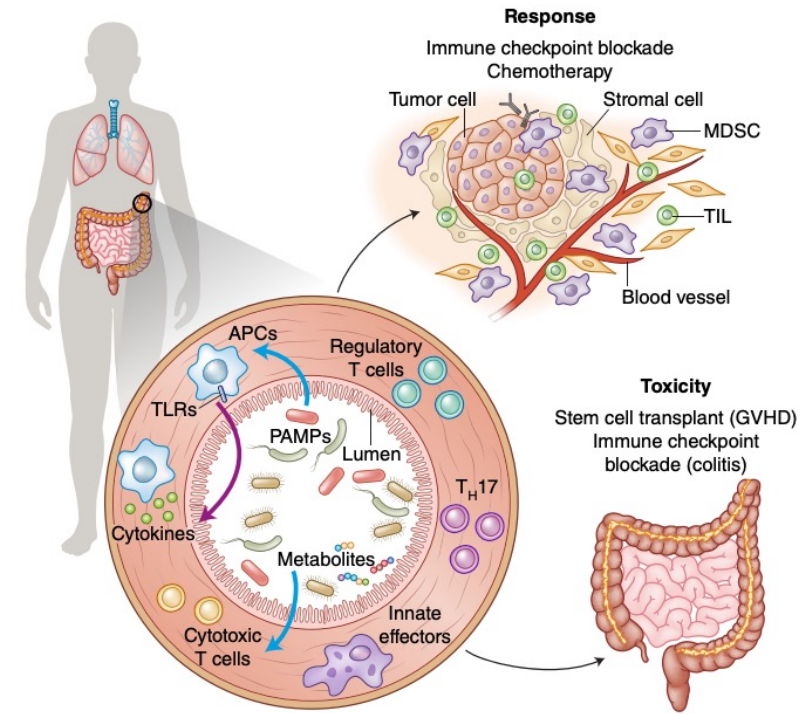
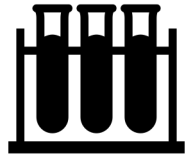


Fig. 2 | The influence of the gut microbiota on different cancer therapies.

Gut microbes can impact both the response to various cancer therapies and associated toxicities, such as colitis and GVHD. The gut microbiota is thought to alter systemic immune function via local changes within the gut mucosa and gut-associated lymphoid tissue. The interaction of PAMPs with APCs and innate effectors via PRRs (TLRs) can help prime an adaptive immune response. Cytokines and microbial metabolites produced locally can act systemically. These combined activities lead to increased antitumor immune function with increased numbers of tumor-infiltrating lymphocytes (TILs) and decreased numbers of myeloid-derived suppressor cells (MDSCs). Credit: Debbie Maizels/Springer Nature.

Basic steps in a microbiome analysis

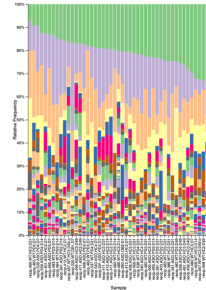
Sample Collection /
Wet lab work



Raw Sequencing Data

```
>NR_024570.1 Escherichia coli strain U  
5/41 16S ribosomal RNA, partial  
sequence  
AGTTTGATCATGGCTCAGATTGAACGCT  
GGCGGCAGGCCTAACACATGCAAGTCG  
AACGGTAACAGGAAG  
CAGCTTGCTGCTTTGCTGACGAGTGGCG  
GACGGGTGAGTAATGTCTGGGAAACTG  
CCTGATGGAGGGGGA.....
```

Statistical Results /
Visualizations



16S rRNA = Bacteria /
Archaea
18S rRNA = microbial
eukaryotes
ITS = Fungi



Sequencing

Quality control

Clustering /
Denoising

Normalization,
Diversity
Assessments



Sequence
Processing / Analysis

Differential
Abundance Testing

Sample
Classification: RF

Longitudinal
Analysis

What is QIIME2?

A powerful, extensible, and decentralized microbiome analysis package with a focus on data and analysis transparency. QIIME 2 enables researchers to start an analysis with raw DNA sequence data and finish with publication-quality figures and statistical results. --- 2016-2021, QIIME 2 development team.



~32,558 citations of QIIME and QIIME2 – Google Scholar, March 2022

***molBV* reveals immune landscape of bacterial vaginosis and predicts human papillomavirus infection natural history**

[Mykhaylo Usyk](#), [Nicolas F. Schlecht](#), [Sarah Pickering](#), [LaShanda Williams](#), [Christopher C. Sollecito](#), [Ana Gradissimo](#), [Carolina Porras](#), [Mahboobeh Safaeian](#), [Ligia Pinto](#), [Rolando Herrero](#), [Howard D. Strickler](#), [Shankar Viswanathan](#), [Anne Nucci-Sack](#), [Angela Diaz](#), [Costa Rica HPV Vaccine Trial \(CVT\) Group](#) & [Robert D. Burk](#) ✉

[Nature Communications](#) **13**, Article number: 233 (2022) | [Cite this article](#)

Research | [Open Access](#) | [Published: 04 January 2022](#)

Urogenital Microbiota: Potentially Important Determinant of PD-L1 Expression in Male Patients with Non-muscle Invasive Bladder Cancer

[Chunxiao Chen](#), [Zehai Huang](#), [Pengcheng Huang](#), [Kun Li](#), [Jiarong Zeng](#), [Yuehui Wen](#), [Biao Li](#), [Jie Zhao](#) ✉ & [Peng Wu](#) ✉

[BMC Microbiology](#) **22**, Article number: 7 (2022) | [Cite this article](#)

578 Accesses | **10** Altmetric | [Metrics](#)



Article | [Open Access](#) | [Published: 28 September 2021](#)

Gut bacteria identified in colorectal cancer patients promote tumourigenesis via butyrate secretion

[Shintaro Okumura](#), [Yusuke Konishi](#), ... [Eiji Hara](#) ✉ [+ Show authors](#)

[Nature Communications](#) **12**, Article number: 5674 (2021) | [Cite this article](#)

13k Accesses | **9** Citations | **76** Altmetric | [Metrics](#)

Gene

Volume 811, 15 February 2022, 146083



Altered cervicovaginal microbiota in premenopausal ovarian cancer patients

[Asuka Morikawa](#)^{a, b}, [Ayako Kawabata](#)^a, [Katsuhiko Shirahige](#)^b, [Tetsu Akiyama](#)^b, [Aikou Okamoto](#)^a, [Takashi Sutani](#)^b ✉

Article | [Open Access](#) | [Published: 30 November 2021](#)

Oral microbiome associated with lymph node metastasis in oral squamous cell carcinoma

[Young-Gyu Eun](#), [Jung-Woo Lee](#), [Seung Woo Kim](#), [Dong-Wook Hyun](#), [Jin-Woo Bae](#) & [Young Chan Lee](#) ✉

[Scientific Reports](#) **11**, Article number: 23176 (2021) | [Cite this article](#)

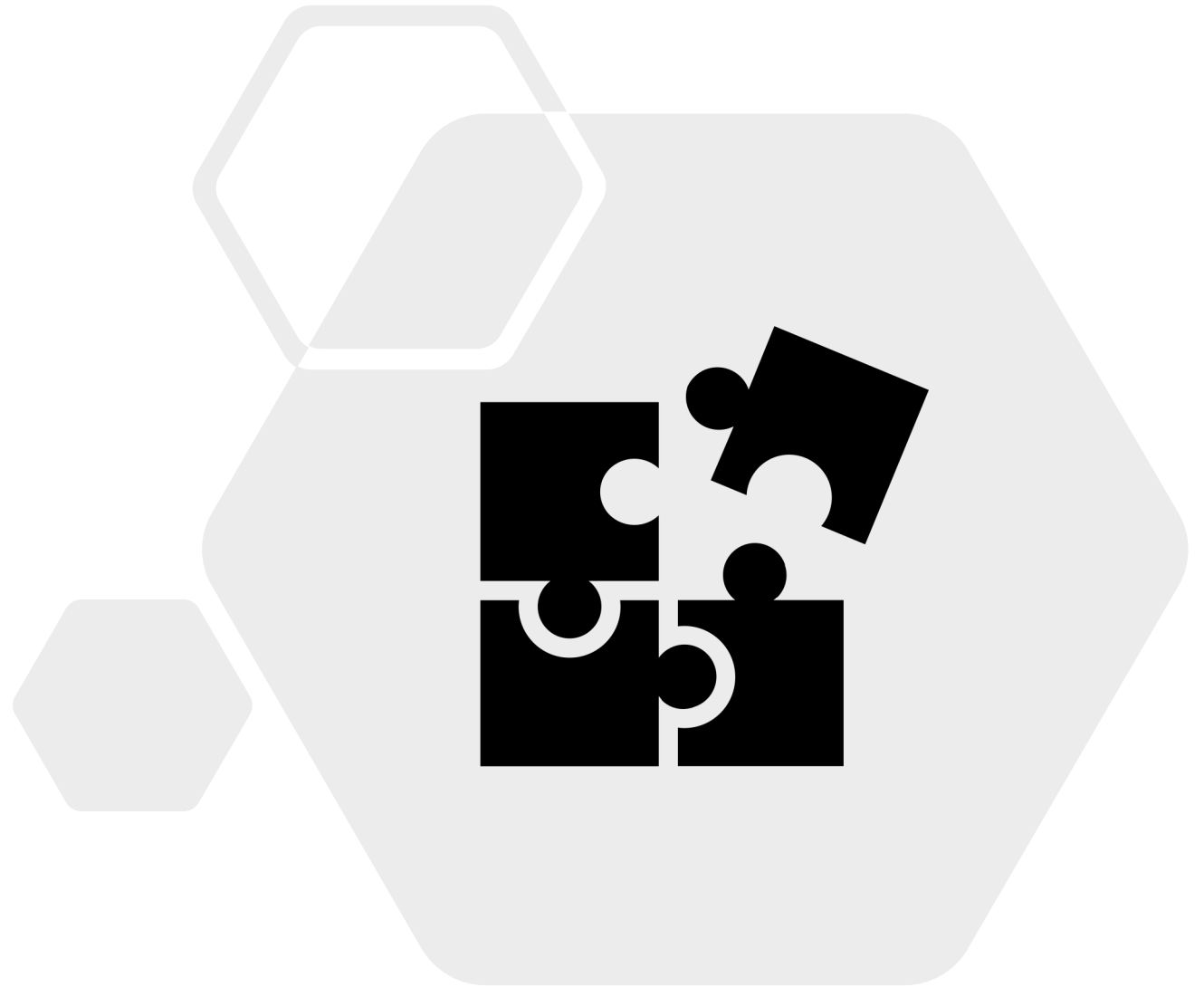
911 Accesses | **1** Citations | **1** Altmetric | [Metrics](#)

Why use Qiime2?

- Methods evolve with the field
 - Plug-in architecture
- Interactive data exploration
- Easy to use and accessible
 - Open-source
 - Extensive documentation
 - Tutorials and workshops
- Widespread use
 - Qiime2 forum
 - Global network of collaborators
- Provenance tracking
 - Facilitates reproducibility
- Multiple user interfaces
 - Galaxy
 - Q2 Studio prototype
 - Python API
 - Command line
 - R API (IN THE WORKS)

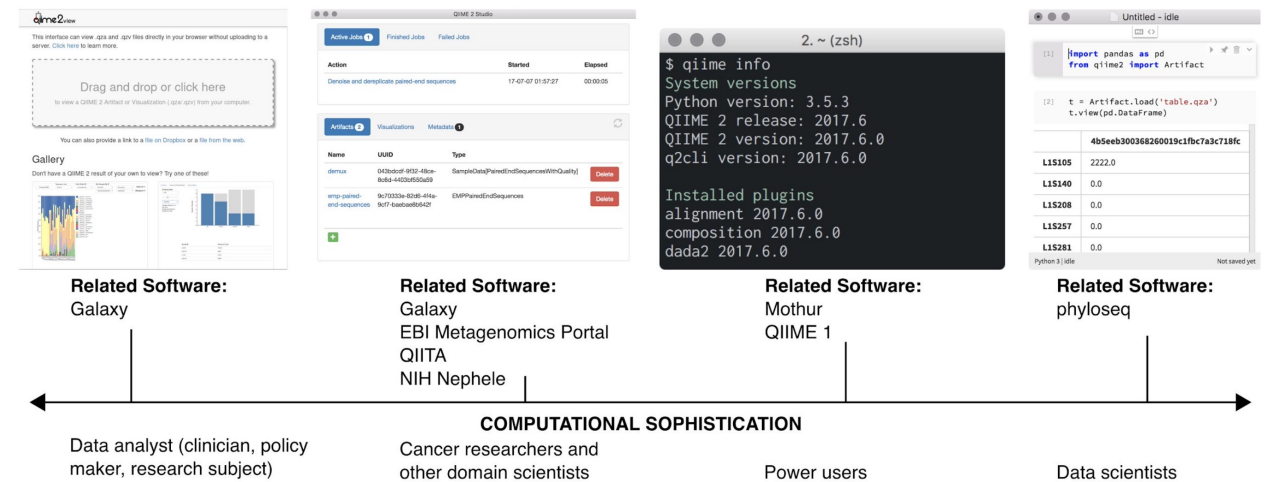
Plug-in Architecture

- Plugins are software packages that can be developed by anyone
- All bioinformatic functionality in QIIME2 defined by plugins
- New plug-ins found in the QIIME2 library



Interfaces

- Q2studio
 - Graphical user interface
- Q2cli
 - Command line interface
 - The classic way to use qiime2
- Artifact API
 - Python 3 application programming interface (API) for QIIME 2
 - Use with Jupyter notebook
 - Recommended for advanced users
- Now on Galaxy
- Potentially coming soon to R
 - For now, qiime2R package



QIIME 2 developers, 2021 workshop content

Qiime2 Documentation



Version: 2022.2 ▼

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Quick search

Go

QIIME 2 user documentation

This site is the official user documentation for QIIME™ 2, including installation instructions, tutorials, and other important information. Visit <http://qiime.org> for information on QIIME™ 1.

Getting started

Check out the [getting started](#) guide to begin using QIIME 2.

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Qiime2 Installation

```
----- /usr/local/lmod/modulefiles -----  
QIIME/1.9.1          QIIME/2-2020.8      qiime/2-2019.4  
QIIME/2-2018.2       QIIME/2-2020.11 (D) qiime/2-2019.10  
QIIME/2-2019.1       QIIME/2-2021.4      qiime/2-2020.2  
QIIME/2-2019.4       qiime/1.9.1         qiime/2-2020.8  
QIIME/2-2019.10      qiime/2-2018.2      qiime/2-2020.11 (D)  
QIIME/2-2020.2       qiime/2-2019.1      qiime/2-2021.4
```

q2cli installation
on macOS, Linux,
and Windows
(WSL)

Qiime2 installed
on Biowulf

module load QIIME

Getting a Biowulf account:


<https://hpc.nih.gov/docs/accounts.html>

Qiime 2 Biowulf help page

<https://hpc.nih.gov/apps/QIIME.html#:~:text=QIIME2%20on%20Biowulf&text=QIIME%20is%20a%20powerful,quality%20figures%20and%20statistical%20results.>

Tools for Reproducibility

Qiime2view



This interface can view .qza and .qzv files directly in your browser without uploading to a server. [Click here](#) to learn more.

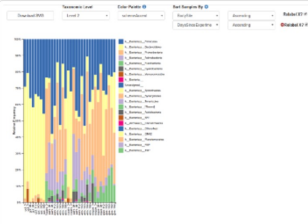
Drag and drop or click here

to view a QIIME 2 Artifact or Visualization (.qza/.qzv) from your computer.

You can also provide a link to a [file on Dropbox](#) or a [file from the web](#).

Gallery

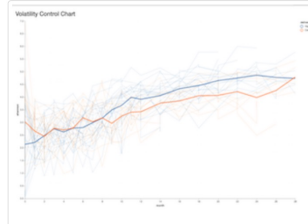
Don't have a QIIME 2 result of your own to view? Try one of these!



Taxonomic Bar Plots

Explore the taxonomy of samples in the Moving Pictures Tutorial. Try selecting different taxonomic levels and metadata-based sample sorting.

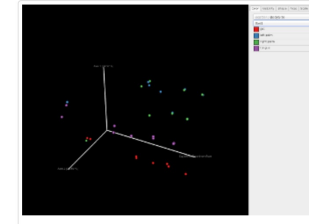
[Try it!](#)



Volatility Control Chart

Explore interactive line plots to assess how volatile a dependent variable is over a continuous, independent variable in one or more groups.

[Try it!](#)



Emperor Ordination

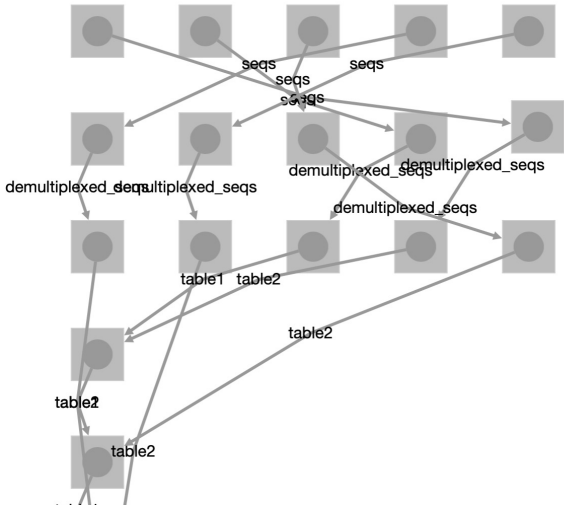
View the differences between sample composition using unweighted UniFrac in ordination space. Color the samples by different metadata columns.

[Try it!](#)

Provenance Tracking

Provenance Graph

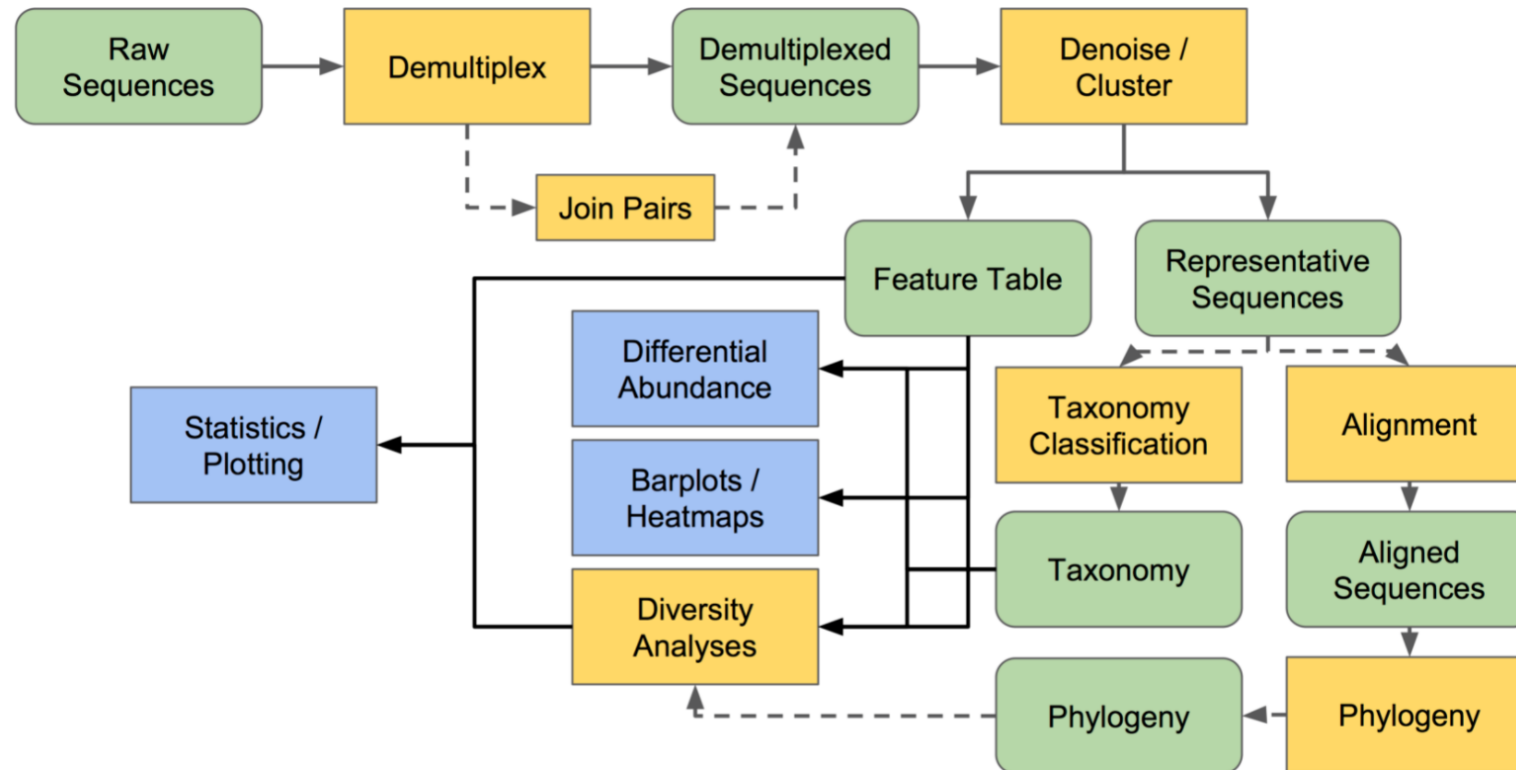
Citations



Details

Click on an element of the Provenance Graph to learn more.

Q2 workflows



<https://docs.qiime2.org/2022.2/tutorials/overview/#let-s-get-oriented-flowcharts>

Core plug-in Overview

<https://docs.qiime2.org/2022.2/plugins/available/>

Available plugins and methods with q2 installation

- Focus on amplicon sequence processing and analysis
 - Primary targets: 16S rRNA, 18S rRNA, ITS
 - QC analysis, adapter / primer trimming via cutadapt
 - OTU clustering, Sequence Denoising
 - DADA2, Deblur, OTU clustering via vsearch (close reference and open reference)
 - Taxonomic Classification
 - Tree building
 - MAFT and fasttree
 - Raxml
 - SEPP fragment insertion
 - Data analysis
 - RF classification, differential abundance testing, core features, alpha diversity, beta diversity, longitudinal methods.

Data import types

- First step = data import
 - Can import at most stages in the analysis
 - qiime tools import -h
 - Raw data usually some type of fastq files
 - EMPPairedEndSequences
 - EMPSingleEndSequences
 - SampleData[SequencesWithQuality]
 - MultiplexedPairedEndBarcodeInSequence
 - RawSequences
- See the import tutorial for help
 - <https://docs.qiime2.org/2022.2/tutorials/importing/>

```
Bowtie2Index
DeblurStats
DistanceMatrix
[EMPPairedEndSequences
EMPSingleEndSequences
ErrorCorrectionDetails
FeatureData[AlignedProteinSequence]
FeatureData[AlignedRNASequence]
FeatureData[AlignedSequence]
FeatureData[Differential]
FeatureData[Importance]
FeatureData[PairedEndRNASequence]
FeatureData[PairedEndSequence]
FeatureData[ProteinSequence]
FeatureData[RNASequence]
FeatureData[SILVATaxidMap]
FeatureData[SILVATaxonomy]
FeatureData[Sequence]
FeatureData[Taxonomy]
FeatureTable[Balance]
FeatureTable[Composition]
FeatureTable[Design]
FeatureTable[Frequency]
FeatureTable[PercentileNormalized]
FeatureTable[PresenceAbsence]
FeatureTable[RelativeFrequency]
Hierarchy
MultiplexedPairedEndBarcodeInSequence
MultiplexedSingleEndBarcodeInSequence
PCoAResults
Phylogeny[Rooted]
Phylogeny[Unrooted]
Placements
ProcrustesStatistics
QualityFilterStats
RawSequences
SampleData[AlphaDiversity]
SampleData[BooleanSeries]
SampleData[ClassifierPredictions]
SampleData[DADA2Stats]
SampleData[FirstDifferences]
SampleData[JoinedSequencesWithQuality]
SampleData[PairedEndSequencesWithQuality]
SampleData[Probabilities]
SampleData[RegressorPredictions]
SampleData[SequencesWithQuality]
SampleData[Sequences]
SampleEstimator[Classifier]
SampleEstimator[Regressor]
SeppReferenceDatabase
TaxonomicClassifier
UchimeStats
```

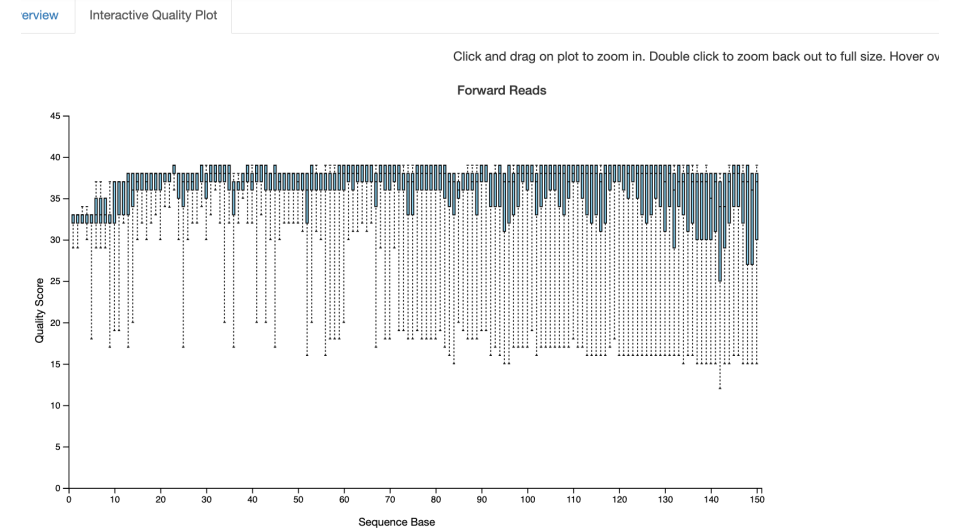
Demultiplex methods & Quality Control

Demultiplex: Associating sequences with samples

- May be done by the sequencing core
- Methods available
 - q2-demux
 - Emp-paired
 - Emp-single
 - q2-cutadapt
 - Demux-paired
 - Demux-single

Quality Control

- Adapter, primer trimming
 - Q2-cutadapt
- Quality score filtering
 - q2-quality-filter
- Removing human contamination
 - Q2-exclude-seqs
 - Q2-filter-reads
- Community evaluation (expected vs observed)
 - Q2-quality-control



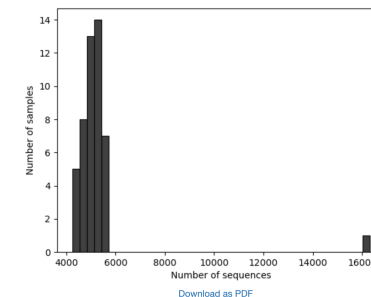
These plots were generated using a random sampling of 10000 out of 253874 sequences without replacement. The minimum sequence length identified during subsampling was 150 bases. Outlier quality scores are not shown in box plots for clarity.

Overview [Interactive Quality Plot](#)

Demultiplexed sequence counts summary

	forward reads
Minimum	4237
Median	5101.5
Mean	5289.041667
Maximum	16327
Total	253874

Forward Reads Frequency Histogram

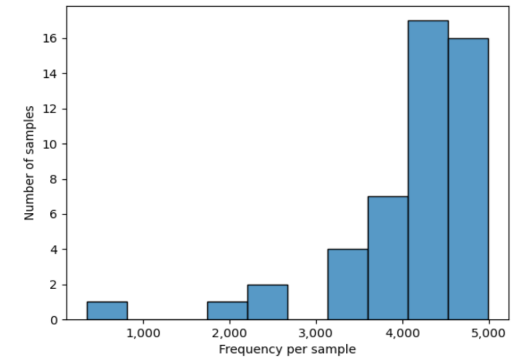
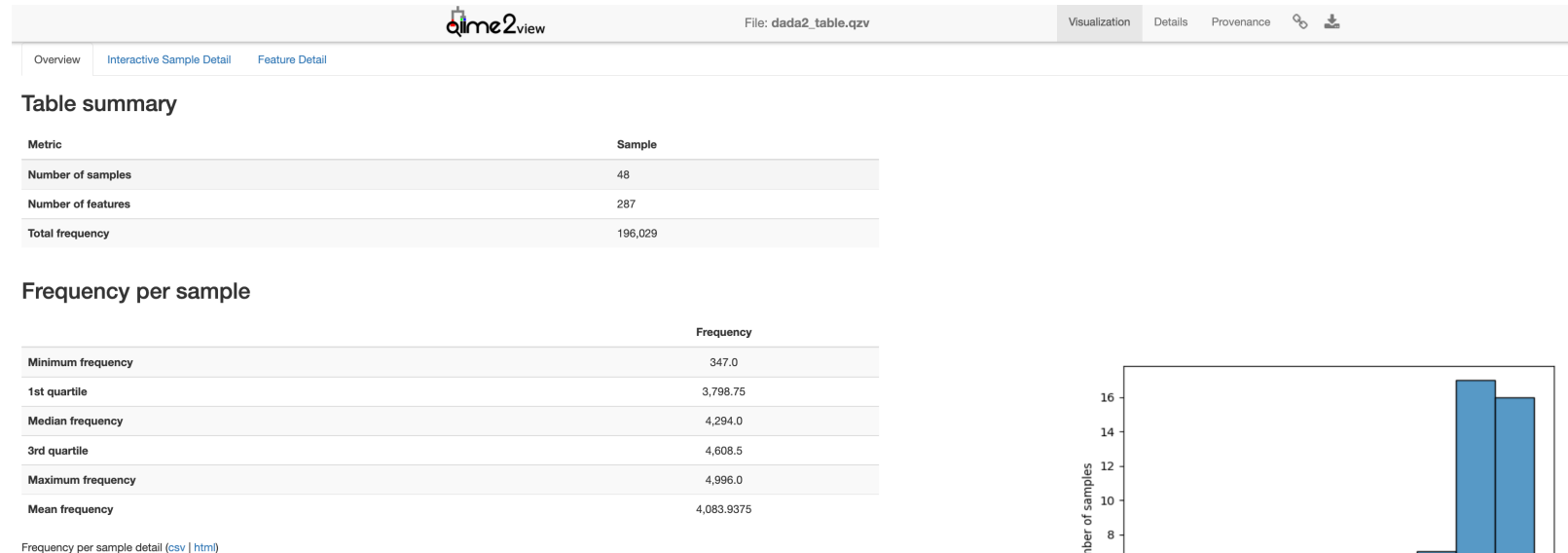


Feature Table generation

- OTU clustering
 - vsearch
- Denoising
 - Deblur
 - DADA2

Outputs: Two key files

1. Feature table
2. Representative Sequences



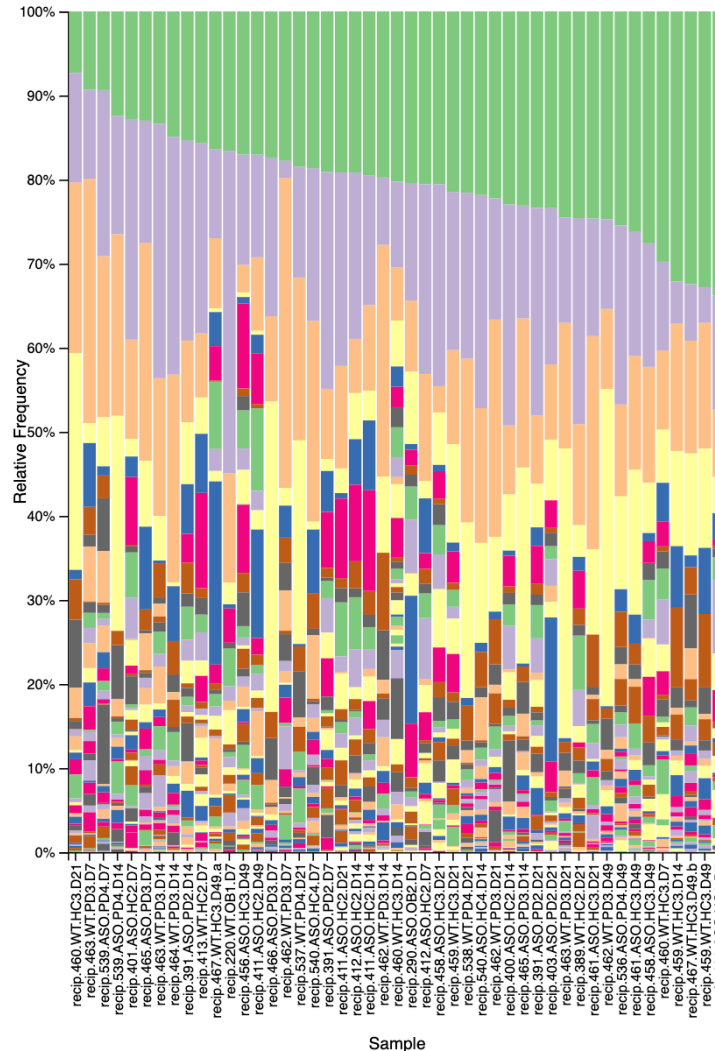
Other feature table methods:

<https://docs.qiime2.org/2022.2/plugins/available/feature-table/>

Taxonomic Classification & Phylogenetic Tree

Taxonomy

- classify-consensus-blast
 - BLAST+ consensus taxonomy classifier
- classify-consensus-vsearch
 - VSEARCH-based consensus taxonomy classifier
- classify-sklearn
 - Pre-fitted sklearn-based taxonomy classifier



Tree

- [Fragment insertion – SEPP](#)
- qiime phylogeny
 - [align-to-tree-mafft-fasttree](#)
 - [align-to-tree-mafft-iqtree](#)
 - [align-to-tree-mafft-raxml](#)

**NO CURRENT WAY TO VIEW TREES
USING QIIME2 PLATFORM**

Community analysis (visualizations / statistics)

qiime diversity core-metrics-
phylogenetic

- [Emperor plots](#)

Alpha and beta group significance
testing

- [output](#)

Differential abundance testing

- [ANCOM](#)


Longitudinal testing

- [Distance based](#)

Data Resources

- Taxonomy Classifiers
- Weighted Taxonomic Classifiers
- Q2 formatted reference marker gene databases
- SEPP reference databases

QIIME2 Plugin Library


library

HomePluginsAboutResources ▾

Latest Plugins


evidentv0.1.0

Effect size and power calculations for microbiome diversity data.




q2-krona1.0.1

Plugin for creating Krona plots.




q2-health-index0.1

QIIME 2 plugin for calculating the Health Index from microbiome data. The plugin is based on the Gut Microbiome Health Index (GMHI) created by Gupta et al. 2020.




q2-dbBact1.1.0

dbBact term enrichment and wordcloud generation for amplicon experiment analysis




q2-sidle2021.02

Taxonomic resolution for marker gene molecules can be improved by encompassing longer sequences. The Short Multiple Reads Framework algorithm allows the scaffolding of multiple marker gene regions against a reference database; this plugin allows the construction of a database, reconstruction, and extends the algorithm and introduces a way to generate a corresponding phylogenetic tree following reconstruction.



q2-data-augment1.0

Data augmentation is a very useful and widely used method in data science. Especially, it can increase the sample size of the training set for machine learning models. Rarefy for Augment uses a simple rarefaction method to achieve data augmentation. The data augmentation should only be implemented on the training data set.



Future Features

- Funding through NCI
 - **To increase the accessibility of the QIIME 2 platform and cancer microbiome data to the cancer research community**
 - Galaxy
 - R API
 - Cancer microbiome data resource via QIITA
 - cancer-focused QIIME 2 user workshop series
 - Collaboration with NCI Cloud Resources teams to integrate QIIME 2
 - Work directly with data from GATK and TCGA
 - **Make QIIME 2 a microbiome multi-omics bioinformatics platform**
 - **Facilitate reproducible research**

[Informatics Technology for Cancer Research](#) program
Project 1U24CA248454-01;
<https://reporter.nih.gov/project-details/9951750>



Future – omics tools

- Integrating multi-omics data
 - Shotgun surveys of phage and other viruses
 - Functional potential via shotgun metagenomics
 - Functional activity
 - Metatranscriptome
 - Metaproteome
 - metabolome



Provenance Replay

- The ability to generate executable workflows from an existing artifact or visualization's data provenance

Other features:

- ability to obtain provenance info in a publication ready format
- Automated workflow bug detection from data provenance
- Plugins to help users submit data to repositories

Qiime2 forum

A vast network of collaborators and users

Help in just a short period of time

all categories ▾

all tags ▾

Categories

Latest

Top

Category

Topics

Latest

User Support

38 / month

Post to this category if you need help understanding output produced while running QIIME 2. Examples of this include help understanding plots labels, techniques that are used in QIIME 2, etc. Posts in this category will be triaged by a QIIME 2 Moderator and responded to promptly.

Technical Support

33 / month

Post to this Category if you are experiencing a technical difficulty while running QIIME 2. Examples of difficulties include installation errors, help deciphering error messages, etc. Posts in this category will be triaged by a QIIME 2 Moderator and responded to promptly.

Community Plugin Support

12 / month

Post to this category if you have a question about a [community plugin](#) (bug report, technical detail, etc.). Community plugins are plugins not distributed in the QIIME 2 Core Distribution. Please note, in the future we are planning on moving away from the notion of a "Core Distribution," where all pl...

General Discussion

18 / month

Post to this category if you have a general question about microbiome science, bioinformatics, or other general questions, ideas, or topics to discuss. Examples of posts include study design, paper discussion, etc. Posts in this category will not be triaged by a QIIME 2 Moderator.

Developer Discussion

3 / month

Post to this category if you are a developer and have a questions, idea, or suggestion. Posts in this category will be triaged by a QIIME 2 Moderator and responded to promptly.

🌱

Frequent Questions and "Best of the QIIME 2 Forum"

General Discussion

best-of-the-forum

2

Feb '20

👤

how to get picrust2 plug in with qiime2 2022.2

Community Plugin Support

picrust

queued

7

8m

🌱

q2-shogun and q2-metaphlan2 plugins

Community Plugin Support

taxonomy

queued

4

1h

👤

Why do I loose 36.8% of reads while using PEAR for merging?

Other Bioinformatics Tools

2

2h

🌱

Databases for known bacteria-disease relationships

General Discussion

queued

0

3h

🌱

Select samples randomly based on columns in metadata?

User Support

1

3h

👤

looking for other conserved genes besides 16S

General Discussion

0

9h

🌱

Metadata file path doesn't exist

Technical Support

metadata

2

10h

Current Learning Opportunities through Qiime2

Interested in hosting or attending an official QIIME 2 Workshop? [Contact us](#).

Upcoming Workshops

No upcoming workshops

Past Workshops

Title	Location	Workshop Dates
BIOF 089 - Microbiome Bioinformatics with QIIME 2	Online (via FAES at the National Institutes of Health)	Jan. 31, 2022 - Feb. 4, 2022
Microbiome Bioinformatics with QIIME 2: Free online workshop	Online	Jan. 26, 2021 - June 1, 2021
Microbiome Bioinformatics with QIIME 2	Online (via FAES at the National Institutes of Health)	Jan. 4, 2021 - Jan. 8, 2021
An Introduction to QIIME 2	Online (via World of Microbiome)	Nov. 4, 2020 - Nov. 4, 2020
CZI-CABANA Microbiome Bioinformatics with QIIME 2 Workshop (sold out)	Online	Oct. 5, 2020 - Oct. 9, 2020
Introduction to microbiome study design and analysis	Puerto Rico	Aug. 1, 2020 - Aug. 1, 2020
Microbiome Bioinformatics with QIIME 2	Bethesda, Maryland	Jan. 8, 2020 - Jan. 10, 2020
Microbiome Bioinformatics with QIIME 2 Workshop	Fort Collins, Colorado (USA)	Nov. 25, 2019 - Nov. 26, 2019
Microbiome Bioinformatics with QIIME 2 Workshop (not open to the public)	University of Wyoming	Oct. 24, 2019 - Oct. 25, 2019
Microbiome Bioinformatics with QIIME 2	Bangkok, Thailand	Sept. 11, 2019 - Sept. 12, 2019
QIIME 2 @ One Health Summer School	University of Bern, Switzerland	Aug. 14, 2019 - Aug. 14, 2019

QIIME2 Youtube Channel

The screenshot displays the QIIME2 YouTube channel interface. On the left is a dark sidebar with navigation links: Home, Explore, Shorts, Subscriptions, Library, and History. Below these is a 'Sign in to like videos, comment, and subscribe.' section with a 'SIGN IN' button, followed by a 'BEST OF YOUTUBE' section with icons for Music, Sports, Gaming, Movies & Shows, News, Live, Fashion & Beauty, Learning, and Spotlight. The main content area features the channel header with the QIIME2 logo, name, and 2.06K subscribers, along with a 'SUBSCRIBE' button. Below the header is a navigation bar with tabs for HOME, VIDEOS, PLAYLISTS, COMMUNITY, CHANNELS, and ABOUT. The first featured section is the 'QIIME 2 Cancer Microbiome Intervention Tutorial (CMIT)' playlist, described as a JupyterBook available at <https://docs.qiime2.org/jupyterbooks/cancer-microbiome-...>. It contains six video thumbnails with titles like 'Introduction to the QIIME 2 Cancer Microbiome...', 'An introduction to QIIME 2', 'Running QIIME 2 inside Galaxy (alpha release...', 'QIIME 2 Galaxy introduction', 'Semantic types and data formats in QIIME 2', and 'Introduction to the Cancer Microbiome Intervention...'. The second featured section is the 'Fundamentals of Bioinformatics' playlist, taught by Professor Greg Caporaso, with a description of its content and a link to his website. It includes six video thumbnails with titles such as 'Biological Information (part 1)', 'Biological Information (part 2)', 'An Introduction to Microbiome Science (part 1)', 'An Introduction to Microbiome Science (part 2)', 'A quick introduction to Python 3 programming (par...', and 'A quick introduction to Python 3 programming (par...'. Each video thumbnail shows a preview image, the video title, the channel name 'QIIME 2', view counts, and upload dates.

New Cancer Microbiome Tutorial using Galaxy!



QIIME 2 Cancer Microbiome Intervention Tutorial

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GETTING STARTED WITH QIIME 2

Getting started with using QIIME 2



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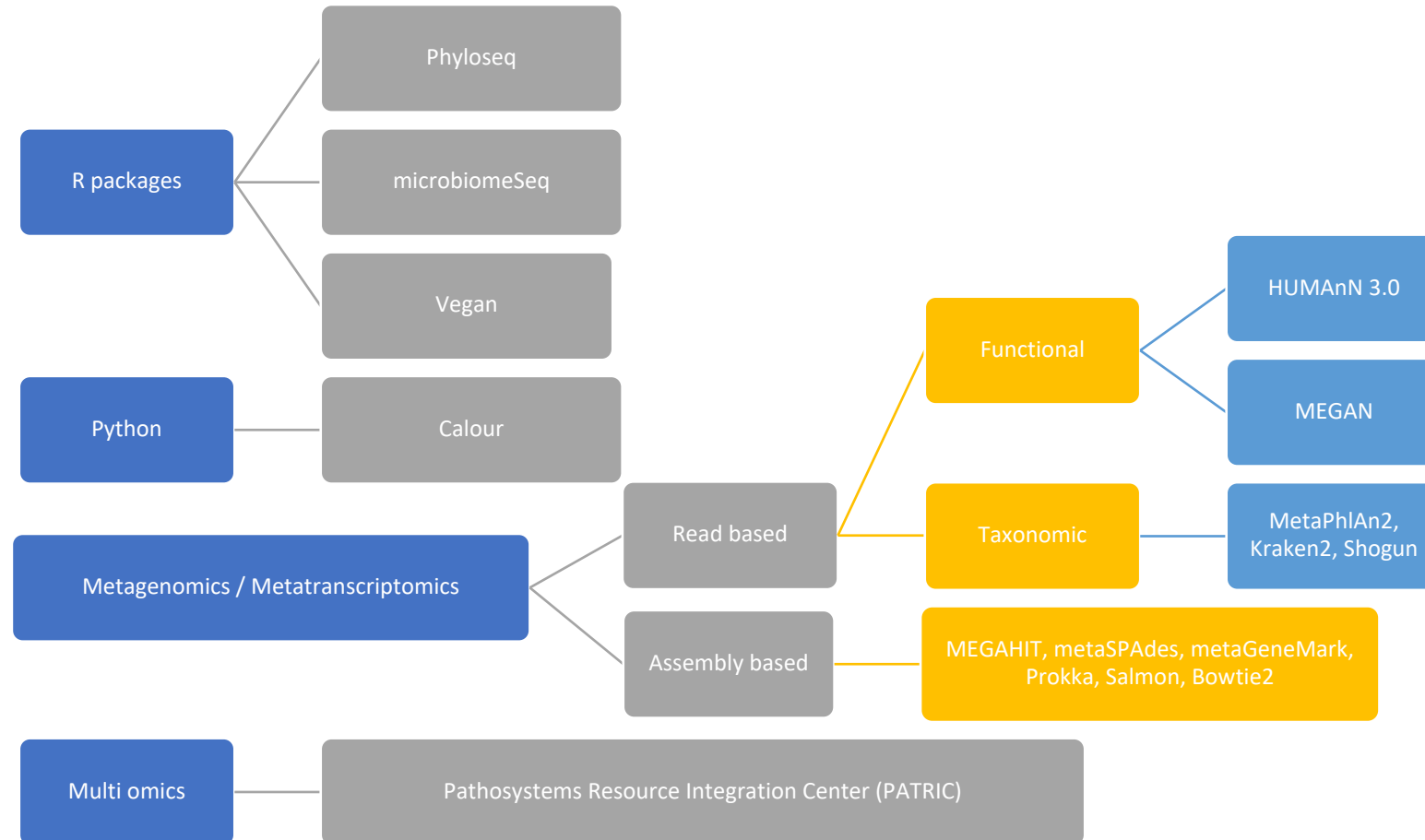
This tutorial was originally developed for the 2022 NIH Foundation for Advanced Education in the Sciences Microbiome Bioinformatics with QIIME 2 workshop, taught online January 31 – February 4, 2022. It is now being publicly released with [corresponding video content](#) on the [QIIME 2 YouTube channel](#).

This tutorial is intended to be read like a book, from beginning to end.

This tutorial and the corresponding videos present many new features of QIIME 2 and our education and documentation ecosystem, including:

- The new QIIME 2 [Galaxy](#)-based graphical user interface;
- New tutorial data;
- Our new QIIME 2 documentation system, which illustrates how to use QIIME 2 with different user interfaces, including Galaxy, the QIIME 2 command line interface, and the QIIME 2 Python 3 API. You can choose to use whichever of these interfaces you're most comfortable with, or user different interfaces for different steps;
- Our first [Jupyter Book](#)-based QIIME 2 tutorial.

Going beyond qiime2



Contact me at ncibtep@nih.gov
for help regarding this resource.